

*What is claimed is:*

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Sub. E3  
1. An isolated nucleic acid molecule comprising an adenovirus tripartite leader (TPL) nucleotide, said TPL nucleotide sequence comprising (a) first and second different TPL exons or (b) first, second and third same or different TPL exons, said TPL exons selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3.

2. The isolated nucleic acid molecule of claim 1, wherein said sequence is operatively linked to an intron containing an RNA processing signal.

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3. The isolated nucleic acid molecule of claim 1 wherein said TPL nucleotide sequence consists essentially of complete TPL exon 1 operatively linked to complete TPL exon 2 operatively linked to complete TPL exon 3.

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4. The isolated nucleic acid molecule of claim 1 wherein said intron is native adenovirus intron 1.

5. The isolated nucleic acid molecule of claim 1 wherein said TPL nucleotide sequence is shown in SEQ ID NO: 32.

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6. The isolated nucleic acid molecule of claim 1 further comprising a promoter and a nucleic acid sequence which encodes an adenoviral structural protein, operatively linked to said promoter and said TPL sequence.

7. The isolated nucleic acid molecule of claim 6 wherein said adenoviral structural protein is a fiber protein or a chimeric protein which includes an adenovirus fiber protein tail domain.

8. The isolated nucleic acid molecule of claim 7 wherein said chimeric protein comprises an Ad3 head domain and an Ad5 tail domain or an Ad5 head domain and an Ad3 tail domain.

5 9. The isolated nucleic acid molecule of claim 7 wherein said molecule is contained in a plasmid selected from the group consisting of plasmids pCLF, pDV60, pDV67, pDV69, pDV80 and pDV90.

10 Sub. E3 10. The isolated nucleic acid molecule of claim 9 wherein said molecule has a nucleotide sequence selected from the group consisting of sequences shown in SEQ ID NO: 8, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 47, SEQ ID NO: 64 and SEQ ID NO: 65..

11. An adenovirus vector complementing plasmid comprising an isolated nucleic acid molecule according to claim 1.

15 Sub. E3 12. An adenovirus vector packaging cell line comprising a stably integrated nucleic acid molecule of claim 1, an operatively-linked promoter and a nucleic acid sequence which encodes an adenovirus structural protein, wherein said TPL sequence consists essentially of a first TPL exon operatively linked to a complete second TPL exon operatively linked to a complete third TPL exon.

Sub. E3 13. The cell line of claim 12 wherein said first TPL exon is a complete or partial first TPL exon.

20 14. The cell line of claim 13 wherein said TPL molecule comprises complete TPL exon 1 having the nucleotide sequence of SEQ ID NO: 32 or partial TPL exon 1 having the nucleotide of SEQ ID NO: 26.

15. The cell line of claim 12 wherein said promoter is an inducible promoter.

16. The cell line of claim 12 wherein said adenovirus structural protein is adenovirus fiber protein or a chimeric protein which includes an adenovirus fiber protein tail domain.

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17. The cell line of claim 12 wherein said chimeric protein comprises an Ad3 head domain and an Ad5 tail domain or an Ad5 head domain and an Ad3 tail domain.

18. The cell line of claim 12 wherein said nucleic acid molecule is selected from the group consisting of plasmids pDV60, pDV67, pDV69, pDV80 and pDV90.

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19. The cell line of claim 18 wherein said nucleic acid molecule has a nucleotide sequence from the group consisting of sequences shown in SEQ ID NO: 43, SEQ ID NO: 44 and SEQ ID NO: 47.

20. The cell line of claim 12 wherein said cell line is an epithelial cell line.

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21. The cell line of claim 20 wherein said cell line supports the production of a recombinant adenovirus vector genome by complementation of a deficient viral gene in said vector genome.

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22. The cell line of claim 21 wherein said cell line further produces an adenovirus protein and thereby complements a deficient adenovirus gene in said vector genome, and wherein said cell line complements an adenovirus early protein gene and a fiber gene.

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23. The cell line of claim 22 wherein the deletion of said deficient adenovirus gene is complemented by the expression of said gene under the control of an inducible promoter.

24. A recombinant adenovirus particle comprising a recombinant adenovirus vector genome wherein said genome does not encode or does not express sufficient adenovirus fiber protein to support packaging of a fiber-containing adenovirus particle without complementation of said fiber gene.

25. The recombinant adenovirus particle of claim 24 wherein said adenovirus vector genome does not encode one or more functional proteins selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4 protein.

26. The particle of claim 24 wherein said adenovirus vector genome is Ad5.Bgal.ΔF.

27. The particle of claim 24 wherein said adenovirus vector genome is contained in the adenovirus particle deposited under ATCC accession # VR2636 and corresponding to Ad5.Bgal.ΔF.

28. The particle of claim 24 wherein said particle lacks fiber protein or contains a modified fiber protein.

29. The particle of claim 24 wherein said particle comprises an adenovirus fiber protein or a chimeric protein having an adenovirus fiber protein tail domain, said chimeric protein comprising an Ad3 head domain and an Ad5 tail domain or an Ad5 head domain and an Ad3 tail domain.

30. The particle of claim 24 wherein said exogenous protein is a therapeutic gene product.

31. A helper-independent fiberless recombinant adenovirus vector genome comprising genes which:

(a) encodes all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to package a fiber-containing adenovirus particle

without complementation of said fiber gene or said genome lacks at least the fibre gene,  
and

(b) encodes an exogenous protein.

5 32. The adenovirus vector genome of claim 31 wherein said adenovirus  
vector genome does not encode one or more functional proteins selected from the  
group consisting of E1A, E1B, E2A, E2B, E3 and E4 protein.

33. The adenovirus vector genome of claim 31 wherein said adenovirus  
vector genome is Ad5.Bgal.ΔF.

10 34. The adenovirus vector genome of claim 33 wherein said adenovirus  
vector genome has a nucleotide sequence shown in SEQ ID NO:27 and corresponds  
to Ad5.Bgal.ΔF.

35. The adenovirus vector genome of claim 31 wherein said adenovirus  
vector genome is contained in the adenovirus particle deposited under ATCC accession  
VR-2636 corresponding to Ad5.Bgal.ΔF.

15 36. The adenovirus vector genome of claim 31 wherein said exogenous  
protein is a therapeutic gene product.

37. An isolated nucleic acid that comprises the adenovirus vector genome  
of claim 31.

20 38. A method for producing an adenovirus vector particle containing a  
helper-independent fiberless recombinant adenovirus vector genome, said method  
comprising providing a packaging cell line which complements replication and  
packaging of said genome and a helper-independent fiberless recombinant adenovirus  
vector genome which is deficient in expressing sufficient functional fiber protein to

support assembly of fiber-containing particles and harvesting said particle produced by said cell line.

39. The method of claim 38 wherein said packaging cell line complements adenovirus fiber protein.

5 40. The method of claim 38 wherein said adenovirus vector genome comprises genes that:

(a) express all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to package a fiber-containing adenovirus particle without complementation of said fiber gene or said genome lacks at least the fibre gene, and

10 (b) express an exogenous protein.

15 Sub B3 41. The method of claim 38 wherein said packaging cell line comprises a stably integrated first nucleic acid molecule alternatively operatively linked to a promoter, and said first nucleic acid is operatively linked to a second nucleic acid molecule encoding an adenovirus structural protein, wherein said first nucleic acid molecule comprises an adenovirus tripartite leader (TPL) nucleotide sequence operatively linked to an intron containing an RNA processing signal, said TPL nucleotide sequence comprising (a) first and second different TPL exons or (b) first, second and third different TPL exons, said TPL exons selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3.

20 42. The method of claim 38 wherein said helper-independent fiberless recombinant adenovirus vector genome is introduced by infecting said cell line with a virus particle containing said genome.

25 43. The method of claim 42 wherein said particle is a particle comprising a helper-independent recombinant adenovirus vector genome comprising genes that:

(a) encode all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to support packaging of a fiber-containing adenovirus particle without complementation of said fiber gene or said genome lacks at least the fibre gene, and

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(b) encode an exogenous protein,

wherein said particle comprises an adenovirus fiber protein or a chimeric protein that includes an adenovirus fiber protein tail domain.

44. The method of claim 38 wherein said helper-independent fiberless recombinant adenovirus vector genome is introduced into said cell line by transfecting said cell line with said helper-independent fiberless recombinant adenovirus vector genome

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45. The method of claim 44 wherein said adenovirus vector genome comprises genes which:

(a) encode all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to package a fiber-containing adenovirus particle without complementation of said fiber gene or said genome lacks at least the fibre gene, and

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(b) encode an exogenous protein.

46. The method of claim 38 wherein said packaging cell line is transfected with a nucleic acid molecule encoding adenovirus fiber protein.

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47. The method of claim 46 wherein said nucleic acid molecule is a nucleic acid molecule comprising an adenovirus tripartite leader (TPL) nucleotide sequence, said TPL nucleotide sequence comprising (a) first and second different TPL exons or (b) first, second and third different TPL exons, said TPL exons selected from the group consisting of complete TPL exon 1, partial TPL exon 1, complete TPL exon 2 and complete TPL exon 3 and said molecule further comprises a sequence encoding adenovirus fiber protein.

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48. The method of claim 39 wherein said adenovirus fiber protein is a modified fiber protein.

49. The method of claim 38 further comprising the step of coating said particle with adenovirus fiber protein.

50. A method for delivery of an exogenous gene to a target cell comprising contacting said cell with an amount of a recombinant adenovirus particle of claim 24 sufficient to infect said cell.

51. The method of claim 50 wherein said exogenous gene encodes a therapeutic gene product.

52. The method of claim 51 wherein said recombinant adenovirus particle contains a modified fiber protein which binds a preselected target cell and directs delivery of the particle to said target cell.

53. The method of claim 50 wherein said recombinant adenovirus particle comprises a helper-independent fiberless recombinant adenovirus vector genome comprising genes that:

(a) encode all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to package a fiber-containing adenovirus particle without complementation of said fiber gene or said genome lacks at least the fibre gene, and

(b) encode an exogenous protein.

54. The method of claim 50 wherein said modified fiber protein has an amino terminal head domain which binds to  $\alpha_v$  integrins and thereby targets cells with  $\alpha_v$  integrin receptors.

55. The method of claim 50 wherein said contacting is conducted *in vitro*.



56. The method of claim 55 wherein said contacting is conducted on cells of a tissue which are first removed from the body of a patient, and the cells are subsequently returned to said patient.

5 57. The method of claim 50 wherein said contacting is conducted *in vivo* by administering said recombinant adenovirus particle to a tissue of said patient.

58. The method of claim 57 wherein said administering is intravenously, intraperitoneally, by aerosol, topically or by injection.

59. A method for pseudotyping recombinant viral vectors comprising complementing a missing fiber gene of a helper-independent or helper dependent fiberless recombinant adenovirus vector genome by expressing in packaging cells a fiber gene from a different adenoviral serotype than said recombinant adenovirus vector, thereby pseudotyping said vector.

60. A method for specifically targeting an adenovirus vector to a cell of choice comprising introducing a helper-independent or helper-dependent fiberless recombinant adenovirus vector genome into a packaging cell line for producing a fiber gene-deleted adenovirus vector, wherein a gene for a missing fiber protein is complemented with a gene for a desired modification for targeting the vector to a cell of choice.

61. A method for producing a modified adenovirus comprising providing *in vitro* an exogenous fiber protein to a fiberless adenovirus.

62. The method of claim 61, wherein said fiber is provided by adding fiber protein in a suitable buffer to a fiberless virus preparation, thereby producing a modified adenovirus.

63. The method of claim 61, wherein a helper-independent or helper-dependent fiberless recombinant adenovirus vector genome is introduced into a packaging cell line to produce a fiberless adenovirus to which exogenous fiber protein will be provided.

5 64. A method for delivering a heterologous gene to EBV-infected B cells comprising infecting said B cells with a pseudotyped Ad5 $\beta$ gal. $\Delta$ F particle or other fiber-deleted adenovirus particle, said particle having a chimeric fiber including the receptor-binding knob domain of the adenovirus type 3 fiber.

10 65. The adenovirus particle of claim 24 wherein said adenovirus vector genome lacks a fibre gene or lacks a portion of the fibre gene sequence such that fibre protein is not expressed in sufficient quantities to support packaging.

66. The recombinant adenovirus particle of claim 24 comprising a helper-independent recombinant adenovirus vector genome comprising genes that:

15 (a) encode all adenovirus structural gene products but do not express sufficient adenovirus fiber protein to support packaging of a fiber-containing adenovirus particle without complementation of said fiber gene or said genome lacks at least the gene encoding fibre, and

(b) optionally encodes an exogenous protein.

20 67. The recombinant adenovirus particle of claim 24 wherein said particle further comprises a nucleic acid encoding an exogenous protein.

25 68. A method for producing a modified adenovirus comprising providing a packaging cell line for producing a fiberless adenovirus helper-dependent fiberless recombinant adenovirus vector genome and a helper virus vector, wherein said cell line complements at least a deficient fiber protein gene, thereby producing the modified adenovirus.

69. The packaging cell line of claim 9 wherein said cell line is selected from the group consisting of 293, A549, W163, HeLa, Vero, 211, 211A and an epithelial cell line comprising the stably integrated nucleic acid molecule.

70. The recombinant adenovirus particle of claim 21 wherein said exogenous protein is selected from a group consisting of a tumor-suppressor protein, a biologically active fragment thereof, a suicide protein and a biologically active fragment thereof.

71. A composition for preparing a therapeutic vector, said composition comprising a plasmid comprising an adenovirus genome lacking a nucleotide sequence encoding a fiber protein or a genome that is incapable of expressing sufficient fiber to result in packaging.

72. A method of delivering a heterologous gene to a human or any animal comprising providing heterologous gene to a target cell wherein said target cell is contacted *in vivo* or *ex vivo* with an amount of a recombinant adenovirus particle of claim 24 sufficient to infect said cell and thereby deliver the heterologous gene.

73. The recombinant adenovirus vector particle of claim 24, wherein no fiber protein is expressed.

74. The recombinant adenovirus vector genome of claim 31, wherein no fiber protein is expressed.

75. The recombinant adenovirus vector particle of claim 24, wherein said genome expresses insufficient fiber to allow incorporation of said protein into the particle such that the particle cannot use the fiber pathway for infection.

76. The recombinant adenovirus genome of claim 31, wherein said genome expresses insufficient fiber to allow incorporation of said protein into a particle such that the particle cannot use the fiber pathway for infection.

77. A method for producing a gutless adenoviral vector particle comprising:

5 a) delivering a helper adenovirus vector genome to an adenovirus vector packaging cell, wherein said helper adenovirus vector genome lacks any gene encoding adenovirus fiber protein or lacks the ability to encode sufficient adenovirus fiber protein to produce an adenoviral vector comprising fiber protein in the absence of complementation by said packaging cell and wherein said packaging cell comprises the nucleic acid molecule of claim 2 operably linked to a promoter and to an adenoviral fiber protein or to a chimeric protein that includes an adenovirus fiber protein tail domain;

10 (b) delivering a gutless adenovirus vector genome to said packaging cell; and

15 (c) recovering the gutless adenoviral vector particle produced by said cell.

78. The method of claim 77, wherein said helper adenovirus vector genome is delivered by viral infection.

79. The method of claim 78, wherein said gutless adenovirus vector genome is delivered by transfection.

80. The method of claim 77, wherein said gutless adenovirus vector genome comprises an operable packaging sequence.

81. The method of claim 80, wherein said helper adenovirus vector genome has a mutation in its packaging sequence that renders said genome substantially incapable of being packaged as an adenoviral vector particle by said packaging cell.

82. The method of claim 80, wherein said helper adenovirus vector genome comprises recombinase sites flanking its packaging sequence and said packaging cell further comprises a nucleotide sequence encoding a recombinase.

83. The method of claim 82, wherein said recombinase site is a lox site and said recombinase is Cre.

84. A helper adenovirus particle comprising an adenovirus vector genome that does not encode or does not express sufficient adenovirus fiber protein to support packaging of a fiber-containing adenovirus particle without complementation of said fiber gene, wherein said genome has a mutation in its packaging sequence that renders said genome substantially incapable of being packaged.

85. The helper adenovirus particle of claim 84, wherein said mutation comprises a deletion of at least one nucleotide in said packaging sequence.

86. The helper adenovirus particle of claim 85, wherein said adenovirus vector genome does not encode functional proteins selected from the group consisting of E1A, E1B, E2A, E2B, E3, and E4 proteins.

87. A helper adenovirus particle comprising an adenovirus vector genome with recombinase sites flanking its packaging sequence, wherein said vector genome does not encode or does not express sufficient adenovirus fiber protein to support packaging of a fiber-containing adenovirus particle without complementation of said fiber gene.

88. The helper adenovirus particle of claim 87, wherein said adenovirus vector genome does not encode functional proteins selected from the group consisting of E1A, E1B, E2A, E2B, E3, and E4 proteins.

89. An adenovirus particle comprising a gutless adenoviral vector genome and a fiberless capsid.

90. An adenovirus particle comprising a gutless adenoviral vector genome and a capsid comprising a modified fiber protein.

5 91. A packaging cell for the production of a fiberless or fiber-modified gutless adenovirus particle comprising an adenovirus vector complementing plasmid and a nucleotide sequence encoding a recombinase, wherein said complementing plasmid comprises the nucleic acid molecule of claim 2 operably linked to a promoter and to a nucleotide sequence encoding an adenoviral fiber protein or a chimeric adenoviral fiber protein.

10 92. The packaging cell of claim 91, wherein said complementing plasmid and said nucleotide sequence encoding a recombinase are stably integrated into the genome of said cell.

15 93. The packaging cell of claim 91, further comprising a helper adenovirus vector genome.

94. The packaging cell of claim 91, wherein said recombinase is Cre..

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